



Mild exposure to fine particulate matter promotes angiogenesis in non-small cell lung carcinoma[☆]

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ABSTRACT

Fine particulate matter (PM_{2.5}) is associated with public health problems worldwide. Especially, PM_{2.5} induces epigenetic and microenvironmental changes in lung cancer. Angiogenesis is important for the development and growth of cancer and is mediated by angiogenic factors, including vascular endothelial growth factor. However, the effects of mild PM_{2.5} exposure on angiogenesis in lung cancer remain unclear. In this study, we examined angiogenic effects using relatively lower concentrations of PM_{2.5} than in other studies and found that PM_{2.5} increased angiogenic activities in both endothelial cells and non-small cell lung carcinoma cells. PM_{2.5} also promoted the growth and angiogenesis of lung cancer via the induction of hypoxia-inducible factor-1 α (HIF-1 α) in a xenograft mouse tumor model. Angiogenic factors, including vascular endothelial growth factor (VEGF), were highly expressed in lung cancer patients in countries with high PM_{2.5} levels in the atmosphere, and high expression of VEGF in lung cancer patients lowered the survival rate. Collectively, these results provide new insight into the mechanisms by which mild exposure to PM_{2.5} is involved in HIF-1 α -mediated angiogenesis in lung cancer patients.

1. Introduction

Lung cancer has been the most common cause of cancer-related deaths worldwide for many years and is characterized by a poor prognosis in its advanced stages (Siegel et al., 2018). Lung cancers are broadly classified into two histological groups: small cell lung carcinoma and non-small cell lung carcinoma (NSCLC) (Sher et al., 2008). NSCLC accounts for the vast majority (almost 85%) of lung cancer cases and has a poor 5-year survival rate (Sher et al., 2008; Siegel et al., 2018).

Lung cancer is caused by many risk factors, such as genetics, smoking, eating habits and exposure to toxins (Ko et al., 2000; Molina et al., 2008; Santos et al., 2021; Thun et al., 2013). Recent studies have shown that environmental air pollution is also an important cause of mortality in patients of lung cancer (Barta et al., 2019; Miranda-Filho et al., 2019). According to a World Health Organization (WHO) report, air pollution is one of the greatest environmental risk factors for global public health (Carlsten et al., 2020). Particulate matter (PM) is a primary pollutant causing ambient air pollution (Landrigan et al., 2018). Toxicological

Abbreviations: DMEM, Dulbecco's modified Eagle's medium; FBS, Fetal bovine serum; HIF-1, Hypoxia inducible factor 1; HMEC-1, Human dermal microvascular endothelial cell 1; NSCLC, Non-small cell lung carcinoma; OS, Overall survival; PM, Particulate matter; TV, Tumor volume; VEGF, Vascular endothelial growth factor; WHO, World Health Organization.

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reports showed that PM with particulates less than 2.5 μm (PM_{2.5}, Fine PM) is more toxic than larger particles (Du et al., 2018; Kim et al., 2018). PM_{2.5} is predominantly from combustion sources such as diesel exhaust and a complex mixture of liquids and small particles such as acids, organic chemicals, metals, or dusts (Li et al., 2018). Short- and long-term exposure to PM_{2.5} is a global public health problem, including respiratory diseases such as asthma and lung cancer, coronary diseases and cardiovascular disease (Andersen et al., 2012; Beelen et al., 2014; Brunekreef and Holgate, 2002; Chen et al., 2018; Hoek et al., 2013; Li et al., 2010; Liu et al., 2021). In particular, PM_{2.5} can penetrate into the lungs through breathing and irritate and injure the alveolar walls, resulting in impaired lung function (Xing et al., 2016).

Angiogenesis is the physiological process by which new blood vessels are formed from the existing vasculature in the earlier stage of vasculogenesis. Angiogenesis plays an essential role in the growth of almost all tumors, including NSCLC. During tumor development, a variety of stimuli, including proangiogenic growth factors can induce angiogenesis (Prager and Poettler, 2012). Vascular endothelial growth factor (VEGF), an essential factor for blood vessel formation (Ferrara, 2009), stimulates the formation of new blood vessels and increases vascular permeability through activation of its receptor VEGFR-1 or VEGFR-2 (Shibuya, 2011). Hypoxia is a powerful inducer of angiogenesis (Walshe and D'Amore, 2008). The factor responsible for angiogenic responses is the transcription complex hypoxia inducible factor 1 (HIF-1). HIF-1 is a transcription factor that regulates the cell response to hypoxia. HIF-1 is a heterodimeric protein composed of HIF-1 α and HIF-1 β subunits. HIF-1 β subunits are constitutive nuclear proteins. However, HIF-1 α is sensitive to oxygen levels (Wang et al., 1995). HIF-1 α consecutively induces angiogenesis by stimulating the transcription of genes encoding angiogenic factors, including VEGF. At last, HIF-1 α and VEGF act as central regulators of angiogenesis and tumor progression in various cancers (Semenza, 2000).

Air pollutants, especially PM_{2.5}, involve highly complicated molecular mechanisms in various diseases, including lung cancer. Evidence has shown that ambient air pollution induces oxidative stress and inflammation in the vascular endothelium and that vascular dysfunction may occur. The present study aimed to investigate the effects of PM_{2.5} on angiogenesis of vascular endothelial cells and consequently on lung cancer progression. In addition, we tried to find changes in the expression of angiogenic factors by ambient air pollution. These factors may predict angiogenesis and cancer progression in the conditions with increasingly serious air pollution.

2. Materials and methods

2.1. Materials

Standard reference material 1649b (urban dust, PM_{2.5}) and YC-1 were purchased from Sigma–Aldrich (St. Louis, MO). HIF-1 α antibodies were obtained from BD Pharmingen (San Diego, CA) and Novus Biologicals (Littleton, CO). Antibodies against α -tubulin, CD31, and VEGF were purchased from Sigma–Aldrich (St. Louis, MO), BD Pharmingen (BD Biosciences), and OriGene Technologies, Inc. (Rockville, MD).

2.2. Cell culture

The human dermal microvascular endothelial cell line (HMEC-1) and human A549 cell line were purchased from the American Type Culture Collection. HMEC-1 cells were incubated in MCDB131 medium (WelGENE, Daegu, Korea) supplemented with 10% fetal bovine serum (FBS, HyClone Laboratories Inc., Logan, UT), penicillin (100 U/ml), streptomycin (100 $\mu\text{g}/\text{ml}$) (Corning, Inc., Corning, NY), 10 ng/ml epidermal growth factor (Sigma–Aldrich) and 1 $\mu\text{g}/\text{ml}$ hydrocortisone (Sigma–Aldrich). A549 cells were maintained in Dulbecco's modified Eagle's medium (DMEM, WelGENE) with 10% FBS, penicillin (100 U/ml), and

streptomycin (100 $\mu\text{g}/\text{ml}$).

2.3. MTT assay

HMEC-1 cells (4.0×10^4 cells per well) and A549 cells (2.0×10^4 cells per well) were plated in a 24-well plate and incubated with various concentrations of PM_{2.5} for 24, 48, 72, and 96 h. Then, MTT assay were performed as described in Supplementary materials and methods.

2.4. Western blot analysis

Cells were harvested and whole cell lysates were lysed using lysis buffer (10 mM Tris–pH 7.4, 150 mM NaCl, and 0.2% NP-40) containing a protease inhibitor cocktail (Sigma–Aldrich). Western blot analysis was performed according to a standard protocol as described in Supplementary materials and methods.

2.5. RT-PCR

A549 cells were exposed to PM_{2.5} (1, 10 $\mu\text{g}/\text{ml}$) for 24 h. Total RNA from the cells was extracted using TRIzol (Invitrogen Life Technologies; Carlsbad, CA) and cDNA was synthesized using MMLV-reverse transcriptase (Beams Biotechnology, Sungnam, Korea) and oligo-dT primers (Sigma–Aldrich). For RT-PCRs, the following primers were used: VEGF 5'-GAG AAT TCG GCC TCC GAA ACC ATG ACT TTC TGT-3' and 5'-GAG CAT GCC CTC CTG CCC GGC TCA CCG C-3', β -actin 5'-GCT CGT CGT CGA CAA CGG CT-3' and 5'-CAA ACA TGA TCT GGG TCA TCT TCT C-3'.

2.6. Wound healing assay

For monolayer culture, HMEC-1 cells (1.0×10^4) were seeded on 4-well plates and incubated for 24 h. Then, the cell layer was scratched with a micropipette tip, and was incubated in fresh medium with 1 mM thymidine (Sigma–Aldrich) to stop proliferation and PM_{2.5} (1, 10 $\mu\text{g}/\text{ml}$) for 24 h. After 24h, the cells were stained with Giemsa (Sigma–Aldrich) and the migrated cells were counted.

2.7. Tube formation assay

Two hundred microliters of Matrigel (BD Pharmingen, San Diego, CA) was polymerized on 24-well plates at 37 °C for 30 min. HMEC-1 cells (1.0×10^5) and A549 cells (5.0×10^4) were mixed, seeded on Matrigel matrix-coated well, and incubated with PM_{2.5} (1, 10 $\mu\text{g}/\text{ml}$) for 24h. The tube formation by HMEC-1 cells was observed using a phase contrast inverted microscope, and quantitative analysis of tube structure was detected by ImageJ software (National Institutes of Health).

2.8. Rat aortic ring sprouting assay

Aortas from 6-week-old SD rats (Daehan Biolink, Chungbuk, Korea) were carefully dissected out, and connective fibroadipose tissue was removed. Aortas were sliced with a thickness of 1 mm and were then placed on Matrigel in 24-well plate and added additional 50 μL of Matrigel. The rings were incubated in MCDB131 medium with 1 and 10 $\mu\text{g}/\text{ml}$ PM_{2.5} for 4 days. Quantitative analysis of the vascular sprouts was performed by ImageJ software.

2.9. Mouse xenograft tumor model

All animal procedures were approved by the Institutional Animal Care and Use Committee protocol approved by the Committee for Care and Use of Laboratory Animals at Kyung Hee University [KHSASP-21-252]. BALB/c-nude mice (6-week-old, male) were purchased from Daehan Biolink (Chungbuk, Korea) and maintained on a 12-h light/12-h dark cycle at 25 °C. For generation of a xenograft tumor mouse model, A549 cells were exposed with 10 $\mu\text{g}/\text{ml}$ PM_{2.5} for 24 h and then, the

cells (5.0×10^6 cells/mouse) were injected into the right or left flank of mice subcutaneously. Mice were assigned to groups with 5 mice for one independent experiment, and the experiments were repeated three times. Mice weights and tumor sizes were measured every day for 4 weeks with a digital balance and Vernier caliper (Mitutoyo Corporation), respectively. After 4 weeks, the mice were sacrificed, and tumors were isolated from the mice. Tumor volume (TV) = $\text{length} \times (\text{width})^2 \times 0.5$.

2.10. Immunofluorescent staining

The A549-derived tumors in mice were sliced with 10 μm depth using a cryostat (Leica Microsystems; Wetzlar, Germany) and placed on gelatin-coated slides. Immunofluorescent staining was performed according to a standard protocol as described in Supplementary materials and methods.

2.11. Colony formation assay

A549 cells were treated with 10 $\mu\text{g}/\text{ml}$ PM2.5 and 20 μM YC-1 for 24 h, and then 500 cells were plated and incubated in 60 mm culture dishes. After 2 weeks, cells were fixed with fixation solution (12.5% acetic acid in methanol) and then stained using crystal violet staining solution (Sigma Aldrich). Number of colonies was counted and plotted.

2.12. Comparisons of gene expression profiles of patients with lung cancer in countries with high and low PM2.5

According to the State of Global Air report (<https://www.stateofglobalair.org/air/pm>), India and China belong to the high PM2.5-exposing group with concentrations ranging from 45 to 85 $\mu\text{g}/\text{m}^3$, Taiwan belongs to the intermediate PM2.5-exposing group with concentrations ranging from 15 to 30 $\mu\text{g}/\text{m}^3$, and Finland and Denmark belong to the low PM2.5-exposing group with concentrations ranging from 0 to 15 $\mu\text{g}/\text{m}^3$. We investigated the annual PM2.5 exposure from 1990 to 2020 for low, intermediate, and high PM2.5-exposing countries in the OECD iLibrary (<https://stats.oecd.org/>). We found statistical differences in PM2.5 exposure levels between low, intermediate, and high PM2.5-exposing countries (Supplementary Fig. 1).

We examined the gene expression profiles of angiogenic factors in patients with lung cancer in high PM2.5-exposing group (China and India), intermediate PM2.5-exposing group (Taiwan), and low PM2.5-exposed group (Denmark and Finland). We found five datasets (a total of 92 cases) (Bertelsen et al., 2019; Kreuz et al., 2021; Singh et al., 2020; Wei et al., 2012; Yuan et al., 2023): GSE168466 for China, GSE138682 for India, GSE27262 for Taiwan, GSE131027 for Denmark, and GSE144338 for Finland (Supplementary Table S1). To correct the batch effect and ethnicity in gene expression profiles of the five datasets, we used the ComBat algorithm from the “sva” package (Carvalho and Iriazary, 2010).

2.13. Survival analysis

We searched the transcriptome datasets using “lung cancer” and “survival” as keywords in GEO to collect NSCLC patients with survival information (Barrett et al., 2012). We downloaded a total of five datasets (GSE14814 (Zhu et al., 2010), 101929 (Mitchell et al., 2017), 61676 (Baty et al., 2017), 50081 (Der et al., 2014), and 37745 (Botling et al., 2013)) (Supplementary Table S2). As a result, 662 samples were obtained. The same preprocessing in the previous section was applied to the datasets. We conducted survival analysis to examine the relationship between VEGFA expression and overall survival (OS). To categorize patients into high and low VEGFA-expressing patient groups, we used the median expression value (9.301) as the cutoff point. Patients were placed in the high VEGFA-expressing group if their expression was equal to or greater than the median and in the low VEGFA-expressing group if

it was lower. Kaplan–Meier plots were generated using the “survival” package in R (version 4.1.0), and the significant difference in survival rates between the two groups was assessed using the log-rank test.

2.14. Statistical analysis

The data are presented as the mean and SEM using SPSS software (v25; IBM Corp) from three independent experiments. Differences between 2 groups were evaluated using unpaired Student's t-test, and differences between 3 groups were evaluated by one-way ANOVA followed by Tukey's post hoc test. $P < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. PM2.5 stimulated angiogenic activities of endothelial cells

HMEC-1 cells were exposed to different concentrations of PM2.5 for 24 h to examine cell viability. As shown in Fig. 1A, PM2.5 at 10 $\mu\text{g}/\text{ml}$ did not affect cell viability, whereas 50 and 100 $\mu\text{g}/\text{ml}$ PM2.5 significantly decreased viability. To monitor the long-term effect of PM2.5 on HMEC-1 viability, we treated cells with 1 and 10 $\mu\text{g}/\text{ml}$ PM2.5 for 96 h. We found that 1 $\mu\text{g}/\text{ml}$ did not show any effect on viability until 96 h and that 10 $\mu\text{g}/\text{ml}$ PM2.5 decreased HMEC-1 viability in a time-dependent manner from 48 h (Fig. 1B). To examine whether PM2.5 with a mild concentration that does not affect cell viability may influence angiogenic activities, we selected 1 and 10 $\mu\text{g}/\text{ml}$ PM2.5 for 24 h for further in vitro studies. Since concentrations above 100 $\mu\text{g}/\text{ml}$ inhibited cell viability, we applied relatively low concentrations compared to previous other studies (Lan et al., 2020; Zhu et al., 2019). To examine the effect of PM2.5 on the angiogenic activities of endothelial cells, first, we tested the migration of HMEC-1 cells using a wounding migration assay. As shown in Fig. 1C, PM2.5 significantly induced the migration of HMEC-1 cells. Moreover, microvessels sprouted from the aortic ring were increased by treatment with PM2.5 (Fig. 1D). These results suggest that relatively mild concentrations of PM2.5 increase angiogenic activities, whereas those do not affect cell viability of endothelial cells.

3.2. PM2.5 increased HIF-1 α -mediated angiogenesis

As a factor that promotes angiogenesis, we examined the effect of PM2.5 on HIF-1 α expression. When HMEC-1 cells were treated with PM2.5, HIF-1 α protein levels were increased (Fig. 2A, Supplementary Fig. 2). Since HIF-1 α is a representative factor that promotes angiogenesis in various cancers, including NSCLC, we also examined HIF-1 α protein levels in A549 cells. As shown in Fig. 2B, HIF-1 α levels were increased by PM2.5 treatment in A549 cells as well as in HMEC-1 cells. Moreover, VEGF, which is a HIF-1 α downstream angiogenic factor secreted from cancer cells, was increased in A549 cells (Fig. 2B, Supplementary Fig. 2). From these data, we assumed that angiogenesis in developing NSCLC can be stimulated by the combined action of vascular endothelial cells and cancer cells mediated by PM2.5. Therefore, tube formation was observed when HMEC-1 cells were mixed with A549 cells under treatment with PM2.5. When PM2.5 was added to HMEC-1 cells mixed with A549 cells, the tube-forming structure was increased (Fig. 2C). These results suggest that PM2.5 absorbed into the lungs can promote angiogenesis in lung cancer.

3.3. PM2.5 increased the tumor growth of A549 cells

To identify the effect of PM2.5 on the tumorigenesis and angiogenesis of NSCLCs, we made a xenograft mouse tumor model. For 4 weeks, there were no differences in body weight between the control and PM2.5 groups (Fig. 3A). However, the volume of the PM2.5-treated tumors increased rapidly compared to that of the control group (Fig. 3B). Four weeks after injection of A549 cells, tumors were isolated and examined.

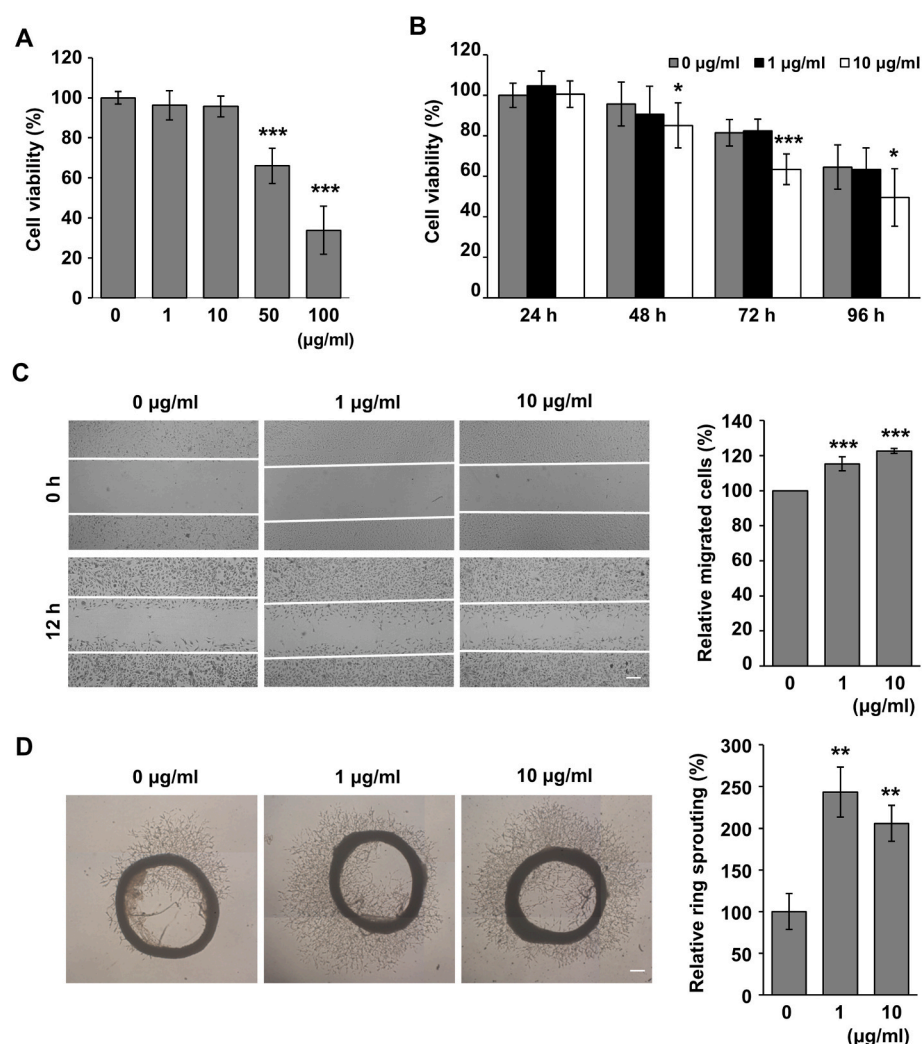


Fig. 1. PM2.5 increased the angiogenic activities of HMECs. (A) HMECs were treated with the indicated concentration of PM2.5 for 24 h. Cell viability was examined using the MTT assay, and the data are presented as the mean $\pm$ SEM. *** p < 0.001 vs. the vehicle-treated control. (B) HMECs were treated with 1 and 10 μ g/ml PM2.5 for the indicated times, and then, viability was determined using the MTT assay. The data are presented as the mean $\pm$ SEM. * p < 0.05; *** p < 0.001 vs. the vehicle-treated control for each time. (C) Representative images of the wounding-migration assay and quantification of the migratory cells beyond reference lines. Scale bar = 250 μ m *** p < 0.001 compared with the control group. (D) Representative images of the rat aortic sprouting assay, and the results were quantified and analyzed statistically. Scale bar = 250 μ m ** p < 0.01 compared with the control group.

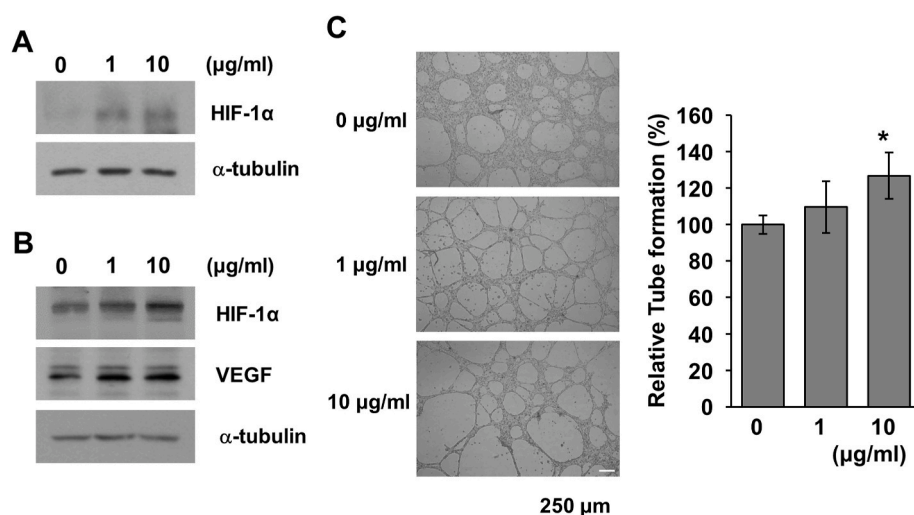


Fig. 2. PM2.5 increased HIF-1 α protein levels in both HMECs and A549 cells. (A) HMECs and (B) A549 cells were treated with PM2.5 for 24 h, and then, whole cell lysates were analyzed by immunoblotting for the indicated proteins. (C) HMECs were mixed with A549 cells at a ratio of 2:1, and mixed cells were seeded on Matrigel and treated with PM2.5. Scale bar = 250 μ m.

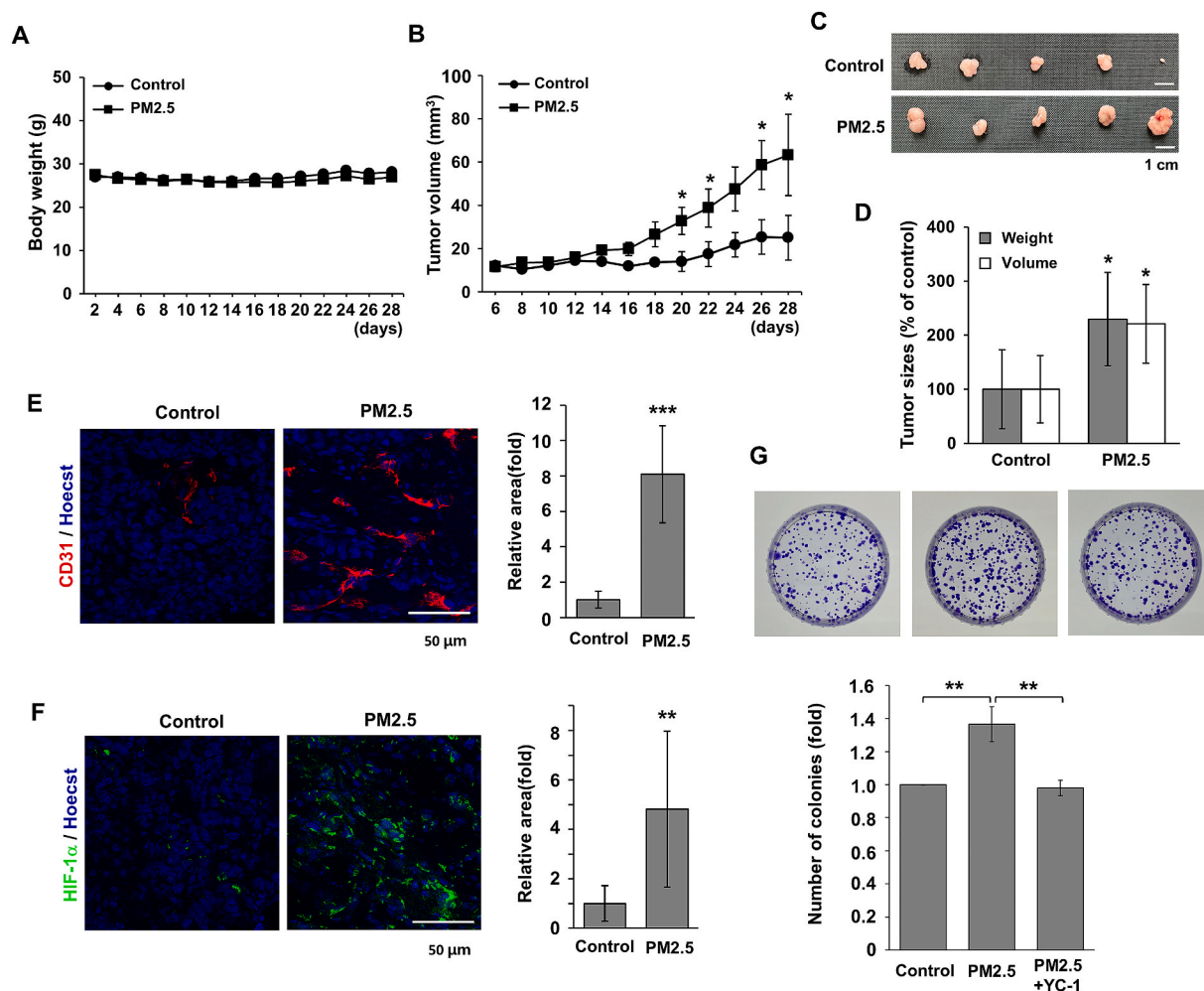


Fig. 3. PM2.5 stimulated tumor growth and angiogenesis in a xenograft NSCLC mouse model. (A, B) BALB/c nude mice were subcutaneously injected with PM2.5-treated A549 cells, and the body weight (A) and tumor volume (B) were measured every day for 4 weeks. The data are presented as the mean $\pm$ SEM. * $p < 0.05$ vs. the control group. (C) Four weeks after injection, the tumors were isolated. Scale bar = 1 cm. (D) Tumor weight and volume were measured. * $p < 0.05$ vs. the control group. (E, F) Microvessels and HIF-1 α were detected in tumor sections by immunofluorescent staining with specific CD31 and HIF-1 α antibodies, respectively. The nuclei were counterstained with Hoechst 33342. Scale bar = 50 μ m. The relative CD31 $^{+}$ and HIF-1 α -expression areas were quantified and plotted from three independent experiments. ** $p < 0.01$, *** $p < 0.001$ versus the control. (G) A549 cells were treated with 10 μ g/ml PM2.5 and 20 μ M YC-1 for 24 h, and then, 500 cells were seeded on 60 mm culture dishes and incubated for 2 weeks. The colony number was counted and plotted from three independent experiments. ** $p < 0.01$.

As shown in Fig. 3C and D, the tumors derived from the PM2.5-treated A549 cells were larger and heavier than those derived from the control cells. To examine whether microvessels inside tumors were increased by PM2.5 treatment, we stained the tissues with CD31 which is a specific marker for endothelial cells. In Fig. 3E, we found that PM2.5 significantly increased the capillaries inside tumors. Moreover, HIF-1 α protein levels in tumor tissues were increased by PM2.5 treatment (Fig. 3F). In order to confirm whether HIF-1 α increased by PM2.5 induced tumorigenesis of NSCLC, we treated YC-1, a specific HIF-1 α inhibitor, and performed a colony formation assay. As shown in Fig. 3G, PM2.5 increased the number of A549 colonies, and the number was significantly decreased by co-treatment with YC-1. From these results, we suggest that PM2.5 induced tumor growth and angiogenesis in NSCLC through HIF-1 α protein.

3.4. Fine dust increased the expression of VEGF in lung cancer patients

The amount of fine dust in the air varies greatly from country to country. To investigate the relationship between the concentration of PM2.5 in the air and the expression of angiogenic factors in lung cancer patients, we classified countries with high and low PM2.5 based on a WHO report. We compared the angiogenesis-related gene expression of

lung cancer patients in China and India (high PM2.5) with those in Finland and Denmark (low PM2.5). As shown by the analysis, the expression of 8 angiogenic factors was significantly higher in lung cancer patients from China and India than in patients from Finland and Denmark (Table 1, Supplementary Fig. 3). In order to overcome the limitations of simple comparison, *VEGFA*, a major factor in angiogenesis, gene was additionally compared by adding database of Taiwan

Table 1

Summary of 8 angiogenic factors significantly increased in countries with high PM2.5

Gene	Product Name	p-value
VEGFA	Vascular endothelial growth factor A	0.0000001502
CCND2	Cyclin D2	0.000008695
JAG2	Jagged Canonical Notch Ligand 2	0.00023
LPL	Lipoprotein Lipase	0.016
PGLYRP1	Peptidoglycan Recognition Protein 1	0.000000008001
SERPINA5	Serpin Family A Member 5	0.049
SLCO2A1	Solute Carrier Organic Anion Transporter Family Member 2A1	0.00055
VAV2	VAV Guanine Nucleotide Exchange Factor 2	0.000002039

$p < 0.05$ was statistically significant.

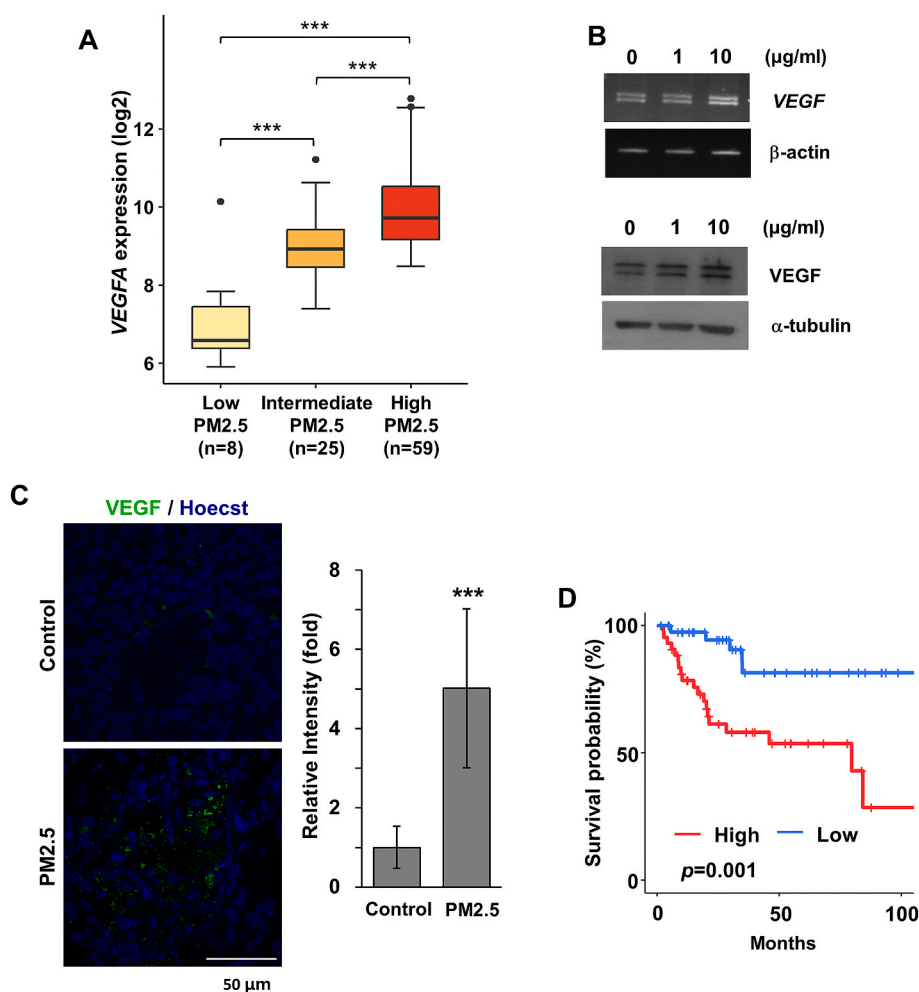


Fig. 4. Correlation of PM2.5 exposure and VEGF expression in lung cancer patients. (A) Boxplot of VEGFA expression in patients with lung cancer in countries with low, intermediate, and high PM2.5. The y-axis represents log2-transformed gene expression values. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. (B) A549 cells were treated with PM2.5 for 24 h, and then, total RNA and whole cell lysates were analyzed by RT-PCR using specific VEGF primer sets (left) and immunoblotting using VEGF antibody (right), respectively, for the indicated proteins. (C) VEGF protein expression was determined in tumor sections by immunofluorescent staining with a specific VEGF antibody. The nuclei were counterstained with Hoechst 33342. Scale bar = 50 μm . The relative intensity of the VEGF fluorescent signal was quantified and plotted from three independent experiments. ** $p < 0.01$ versus the control. (D) Kaplan–Meier survival curve of patients with NSCLC according to VEGFA expression is depicted.

(intermediate PM2.5). VEGFA expression increased significantly in patients with lung cancer as PM2.5 exposure increased (Fig. 4A). VEGFA expression in patients with lung cancer from the intermediate PM2.5-exposing group was significantly higher than those from the low PM2.5-exposing group ($P = 5.61 \times 10^{-5}$). In addition, the expression in patients with lung cancer from the high PM2.5-exposing group was significantly higher than those from the low and intermediate PM2.5-exposing group ($P = 9.99 \times 10^{-9}$, $P = 0.0003$, respectively). Since the expression of HIF-1 α was increased by PM2.5 in the previous experiments, we examined VEGF expression under treatment with PM2.5. As shown in Fig. 4B and Supplementary Fig. 4, both the mRNA and protein levels of VEGF were increased in A549 cells treated with PM2.5. Moreover, in the PM2.5-treated tumor-bearing mice, VEGF protein was also increased in tumor tissues (Fig. 4C). Since VEGF is known to be a factor that impairs prognosis in various types of cancer, it was also confirmed in lung cancer patients. The survival analysis showed that high vs. low VEGFA expression was significantly associated with poor prognosis in NSCLC patients (Fig. 4D). These results suggest that increased VEGF expression by exposure to PM2.5 consequently causes a poor prognosis in lung cancer patients.

4. Discussion

Endothelial cells are cells that form the inner surface of blood vessels, and blood vessels are connected to all organs and tissues of the human body. Therefore, the effects of PM2.5 on endothelial cells can be involved in maintaining homeostasis and inflammation throughout the body. PM2.5 exposure has been demonstrated to be strongly and

robustly associated with an increase in cardiovascular disease risk and endothelial injury (Davel et al., 2012; Pope et al., 2004; Pope et al., 2006). Searching at previous reports investigating the effects of PM2.5 on vascular endothelial cells, PM2.5 induces endothelial dysfunction and vascular inflammatory activities (Davel et al., 2012; Kampfrath et al., 2011; Nurkiewicz et al., 2006). Moreover, based on the results of endothelial cell lines, PM2.5 decreased viability, migration and tube formation, thereby significantly inhibiting angiogenesis (Chen et al., 2017). Most of the previous research papers on PM2.5 applied concentrations much higher than the concentration of PM2.5 in the atmosphere. In fact, it is almost impossible to apply the concentration of PM2.5 in the atmosphere to experiments on cultured cells in the laboratory. When PM2.5 in the air enters the living body through respiration and affects cells and tissues, the actual concentration is expected to be lower. Therefore, in this study, a lower concentration than the concentration applied in other papers was used.

We, for the first time, found that 1–10 $\mu\text{g}/\text{ml}$ PM2.5 increased the migratory and sprouting activities of endothelial cells (Fig. 1 C and D), ultimately promoting angiogenesis. However, contrary to our results, PM2.5 decreases angiogenesis including survival, migration, and tube formation of endothelial cells (Lan et al., 2020; Zhu et al., 2019). They used concentrations higher than 25 $\mu\text{g}/\text{ml}$ (25–800 $\mu\text{g}/\text{ml}$), and we also confirmed that 50 $\mu\text{g}/\text{ml}$ of PM2.5 inhibited the migration of endothelial cells (Supplementary Fig. 5). Although the exact concentration at which the results differ cannot be determined, the results are different according to the high and low concentrations in in vitro conditions. However, for such a conclusion, the effect of various concentrations of PM2.5 on atmospheric concentration levels must be verified.

Because humans breathe in air, PM_{2.5} which is the major pollutant of the atmosphere has been demonstrated to directly affect the incidence of respiratory system disease (Yang et al., 2017). PM_{2.5} is an important cause of progression and mortality in lung cancer worldwide. Many studies on the effects of PM_{2.5} on lung cancer have been reported. PM_{2.5} exposure induces the growth and metastasis of lung cancer by enhancing the miR-802/Rho family GTPase 3 pathway (Li et al., 2016) and increasing lncRNA loc146880 via reactive oxygen species (Deng et al., 2017). Additionally, PM_{2.5} elevates inflammation via the induction of human bronchial epithelial cells and macrophages to release various proinflammatory cytokines, including IL-6, TNF- α and granulocyte-macrophage colony stimulating factor (Baulig et al., 2007; Gualtieri et al., 2008; Li et al., 2020; Zhou et al., 2015). However, although PM_{2.5} exposure has been shown to be associated with angiogenesis, which plays an essential role in the growth and development of lung cancer, the effect of PM_{2.5} on angiogenesis in lung cancer remains unclear. In the present study, PM_{2.5} increased the secretion of VEGF in A549 cells, which then upregulated the expression and transcriptional activity of HIF-1 α , thereby promoting angiogenesis in HMEC-1 cells (Fig. 2). We also found that the capillary and HIF-1 α protein levels in tumor tissues were significantly increased by PM_{2.5} treatment (Fig. 3E and F), and inhibition of HIF-1 α decreased tumorigenicity of A549 cells (Fig. 3G). According to the previous report, VEGF expression and secretion were increased from PM_{2.5}-induced macrophages (Li et al., 2020). Secreted VEGF can promote the angiogenic activities of endothelial cells by binding to VEGF receptor and stimulating angiogenic pathways (Eichmann and Simons, 2012; Sivaraman Siveen et al., 2017). With our experimental model and results, a role of macrophages during lung cancer tumorigenesis and angiogenesis can be ruled out at least. PM_{2.5} directly increased HIF-1 α protein level in NSCLCs and endothelial cells, activated HIF-1 α , which functions as a critical transcription factor for various angiogenic factors and oncogenes (Chan et al., 2005; Melillo, 2007; Semenza, 2010; Wang et al., 1995), may actively promote tumorigenesis and angiogenesis. Because inhibition of HIF-1 α significantly decreased tumorigenicity stimulated by PM_{2.5}, we proved that HIF-1 α is involved in PM_{2.5}-mediated tumorigenesis of NSCLCs.

PM_{2.5} has been reported to increase ROS production in vascular endothelial cells (Shi et al., 2017; Yang et al., 2015) and in chronic obstructive pulmonary disease mice by Nrf2/HO-1 pathway (Zhao et al., 2016). In addition, exposure of PM_{2.5} to A549 cells reduces the activity of catalase and superoxide dismutase, which are antioxidant enzymes, and increases ROS production. ROS can be induced by various stimuli, such as angiotensin II, HIV-1 infection, thymosin beta-4 and insulin, and induced ROS leads to ROS-mediated HIF-1 α stabilization through a Fenton reaction and inhibiting prolyl hydroxylase domain enzymes (Biswas et al., 2013; Duette et al., 2018; Oh and Moon, 2010; Patten et al., 2010; Pagé et al., 2008). Stable HIF-1 α interacts with HIF-1 β , is translocated into nucleus, and activates target genes including VEGF ((Wang et al., 1995; al.; Semenza, 2000).

We further explored the correlation of PM_{2.5} and tumor angiogenesis in lung cancer through analysis of the lung cancer patient database. Interestingly, PM_{2.5} was positively correlated with 8 angiogenic genes in lung cancer patients. These data suggest that PM_{2.5} may promote growth and malignancy in lung cancer by triggering angiogenesis. In order to accurately demonstrate the increase in VEGF expression in lung cancer patients caused by PM_{2.5}, we compared the amount of VEGF expression in lung cancer patients in countries with high, intermediate and low PM_{2.5} and found that VEGF expression was positively correlated with PM_{2.5} concentrations. As in other types of cancer patients (Jung et al., 2021; Zhang et al., 2021), lung cancer patients with higher VEGF expression showed a poor prognosis. When considering overall, the survival rate of lung cancer patients in countries with high PM_{2.5} concentrations is expected to be lower than that in countries with low PM_{2.5} concentrations. Analysis of the survival rate and prognosis of lung cancer patients according to the concentration of PM_{2.5} should be proved clearly, and since PM_{2.5} shows a correlation with the expression

of VEGF, the identification will act as important information for preventing and treating lung cancer against PM_{2.5}.

5. Conclusion

In summary, mild exposure to PM_{2.5} increased the secretion of VEGF by inducing HIF-1 α in A549 NSCLC cells and promoted the angiogenic activities of endothelial cells. Furthermore, PM_{2.5} stimulated tumor progression and angiogenesis in a xenograft mouse model. VEGF expression was high in lung cancer patients in countries with high concentrations of PM_{2.5} in the air, which is closely related to the malignancy of lung cancer.

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Authorship contributions

Joo-Won Jeong designed the experiments; Su Min Ji, Ji Young Lee, Jae-Sun Choi, Ye Won Jang and Sungyeon Kim performed the experiments and analyzed the data; Ja-Eun Kim, Seung Hyeun Lee and Seun-gyoon Nam confirmed and reanalyzed the data Joo-Won Jeong wrote the paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2023.121715>.

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